

CLAIMS

1. Universal polypeptidic carrier for targeting directly or indirectly a molecule to Gb3 receptor expressing cells and having the following formula STxB-Z(n)-Cys, wherein:

- STxB is the Shiga Toxin B subunit or a functional equivalent thereof,

- Z is an amino-acid devoided of sulfydryl group, n being 0, 1 or a polypeptide,

- Cys is the amino-acid Cysteine.

2. Universal carrier according to claim 1 wherein n is 0.

3. Universal carrier according to claim 1 or 2 wherein the molecule is selected in the group constituted of proteins, peptides, oligopeptides, glycoproteins, glycopeptides, nucleic acids, polynucleotides, or a combination thereof.

4. Universal carrier according to claim 1 or 2 wherein the molecule is covalently linked to the -S residue of the universal carrier by a -S-S-, or -S-CO-, or -S-CH₂-. Or -S-NH- linkage.

5. Universal carrier according to claim 4 wherein the molecule is an antigen to be targeted to antigen presentating cells.

6. Universal carrier according to claim 1 or 2 wherein the universal carrier is covalently linked to an oligopeptide or a polypeptide by a -S-S-, or -S-CO-, or -S-CH₂- or -S-NH- linkage, and the molecule to be targeted is operably linked to the said oligopeptide or polypeptide.

7. Universal carrier according to claim 6 characterized in that it is covalently linked to a poly-lysine oligopeptide and the molecule to be targeted is a nucleic acid or an oligonucleotide operably linked to the said poly-lysine moiety.

8. Universal carrier according to claim 4 wherein the molecule is a cytotoxic drug or pro-drug to be targeted to tumor cells expressing Gb3 receptor.

9. An isolated polynucleotide selected from the group consisting of:

a) a polynucleotide comprising the nucleotide sequence STxB encoding the Shiga Toxin B subunit or a functional equivalent thereof bearing at its 3'end the codon TGT, or the codon TGC encoding Cysteine,

b) a polynucleotide comprising a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence encoding the Shiga Toxin B subunit or a functional equivalent thereof bearing at its 3'end the codon TGT or TGC,

c) a nucleotide sequence complementary to the sequence in a) or b).

10. A polynucleotide according to claim 9 having the SEQ ID NO 2.

11. A recombinant vector, or plasmid, comprising a polynucleotide sequence according to claim 9 or 10, for the expression of the universal vector of claim 1 in an appropriate host cell.

12. A recombinant cell line obtained by transformation with the recombinant vector according to claim 11.

13. A recombinant cell line according to claim 12 being a procaryotic cell line, preferably E. coli.

14. A recombinant cell line according to claim 11, 12 or 13,
5 deposited at CNCM on December 19, 2000, with the registration number I-2604.

15. A method for constructing a recombinant vector according to claim 11 or 12 comprising:

10 a) providing a plasmid comprising STxB sequence;
b) applying two PCR amplification steps using two couples of primers, AA' and BB',

- A and B being complementary to each other and comprising the Cys codon,

15 - A' and B' being outside the STxB sequence;

c) isolating the amplified fragments;

d) hybridizing the amplified fragments;

e) applying a PCR amplification on the hybridized fragments;

f) insertion of the amplified fragment into a plasmid.

20 16. A method according to claim 15 wherein in step f) the fragment is inserted into the SphI and Sall restriction site of the plasmid pSU108.

25 17. A process for producing a purified polypeptide according to claim 1 comprising:

a) culturing the cell line according to anyone of claim 12 to
14,

b) obtaining a periplasmic extract of said cells

30 c) purifying said polypeptide.

18. A process according to claim 17 wherein in a), the cell line is E.coli and in c) the purification is made by anion exchange column chromatography followed by a gel filtration column chromatography.

5 19. Method for delivering an sequence of interest into the MHC class I and MHC class II pathway using the a product obtained by covalent binding of the Cys moiety of the universal carrier with said sequence of interest.

10 20. Method for delivering an expression vector containing a sequence of interest into a Gb3 receptor expressing cells characterized in that said expression vector is operably linked to a lysine-rich peptide covalently linked to the Cys moiety of the universal carrier.

15 21. Method according to claim 20 wherein the lysine rich peptide is a 16-mer poly-lysine.

 22. Method according to claim 19 to 21 wherein the sequence of interest is selected amongst:

- 20 - a sequence encoding an immunogenic peptide, or
 - a sequence encoding a drug or a pro-drug becoming toxic for the Gb3 receptor expressing cells, or
 - a sequence encoding a therapeutic active molecule.

25 23. Pharmaceutical composition for enhancing the immunogenicity of a peptide or a protein or a glycoprotein or a lipopeptide, containing the polypepidic carrier according to claim 1 or 2 covalently linked by its Cys moiety to said peptide or protein or glycoprotein or lipopeptide.

30 24. Pharmaceutical composition for treating tumor cells bearing the Gb3 , containing the polypeptidic carrier according to claim 1 or

2 covalently linked by its Cys moiety to a drug or a prodrug toxic for said tumor cells.